INTERACTIONS OF *PYTHIUM MYRIOTYLUM* WITH *FUSARIUM SOLANI*, *RHIZOCTONIA SOLANI*, AND OTHER FUNGI, AND WITH *MELOIDOGYNE ARENARIA* IN PEANUT POD ROT AND PREEMERGENCE DAMPING-OFF

BY

ROBERTO GARCIA

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1974

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Dr. David J. Mitchell, Chairman of the Supervisory Committee, for his counsel, understanding, and encouragement throughout the study and preparation of this manuscript. Thanks also go to the other members of the Supervisory Committee: Dr. L. H. Purdy, Chairman of the Plant Pathology Department, Dr. N. C. Schenck, Dr. R. T. McMillan, and Dr. G. C. Smart for their valuable suggestions and criticisms of this work.

I am indebted to the Food and Agriculture Organization of the United Nations, to the Department of Plant Pathology, University of Florida, and to the Mexican Institution Consejo Nacional de Ciencia y Technologia, for their generous financial support that made this work possible.

Special gratitude is expressed to my wife, Ana Maria, for her encouragement, and her limitless patience and help during my time as a graduate student.

TABLE OF CONTENTS

PAGE

ACKNOWLEDGMENTS ii

LIST OF TABLES iv

ABSTRACT vi

PART 1

Introduction 1
Materials and Methods 4
Results 10
Discussion 21

PART 2

Introduction 25
Materials and Methods 27
Results 33
Discussion 46

LITERATURE CITED 52

BIOGRAPHICAL SKETCH 56

LIST OF TABLES

		Page
1	Percentage of preemergence damping-off of Florunner peanut seedlings after exposure for 3 wk to various pathogens alone or in combinations at defined inoculum densities in infested soil diluted with sterile soil	14
2	Percentage of preemergence damping-off of Florunner peanut seedlings exposed for 3 wk to different pathogens alone or in combinations in soil to which known quantities of reproductive structures were added	15
3	Height, width of largest leaf, and plant volume of Florunner peanut seedlings exposed for 3 wk to various pathogens alone or in combinations at defined inoculum densities in infested soil diluted with sterile soil	16
4	Height, width of largest leaf, number of leaves, and plant volume of Florunner peanut seedlings exposed to various pathogens alone or in combinations in soil to which known quantities of reproductive structures were added	17
5	Recovery of Pythium myriotylum, Rhizoctonia solani, and Fusarium solani from the root system of Florunner peanut plants exposed for 3 wk to inoculum densities prepared by diluting infested soil with autoclaved soil	18
6	Recovery of Pythium myriotylum, Rhizoctonia solani, and Fusarium solani from the root systems of Florunner peanut plants exposed for 2 weeks to soil infested with known quantities of reproductive structures	19
7	Recovery of Pythium myriotylum, Rhizoctonia solani, and Fusarium solani from soil after 3 wk of exposure of Florunner peanut seedlings to defined inoculum densities prepared by diluting infested soil with autoclaved soil or by directly infesting soil with known numbers of reproductive structures.	20

Page

8 Incidence of pod rot and frequency of isolation of fungi from attached peanut pods grown in test tubes containing soil with defined inoculum densities of pathogens established by diluting infested soil with autoclaved soil 39

9 Pod rot severity and frequency of isolation of fungi from attached peanut pods grown in autoclaved soil for 6 wk and then exposed for 20 day periods to soil directly infested with known quantities of reproductive structures of Pythium myriotylum or Rhizoctonia solani 40

10 Pod rot severity and frequency of isolation of fungi from mature detached peanut pods initially grown in autoclaved soil for 6 wk, and then exposed to directly infested soil with known quantities of reproductive structures of Pythium myriotylum or Rhizctonia solani 41

11 Incidence of pod rot and frequency of isolation of fungi from detached mature peanut pods after 8 wk growth in autoclaved soil and 12 days of exposure to combinations of fungi at high and low inoculum levels in soil directly infested with known quantities of the fungal reproductive structures 42

12 Incidence of pod rot and frequency of isolation of fungi from detached peanut pods after 8 wk growth in autoclaved vermiculite and 12 days of exposure to combinations of fungi at high and low inoculum levels in soil directly infested with known quantities of the fungal reproductive structures 43

13 Influence of successive 36 h exposures of detached peanut pods to Petri dish cultures of fungi on pod rot severity, and frequency of isolation of fungi 44

14 Effect of in vitro inoculation of detached peanut pods for 72 h followed by two successive 4 day exposures of pods to soil infested with various fungal pathogens on severity of pod rot and frequency of isolation of fungi from mature pods initially grown in autoclaved soil 45

Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy

INTERACTIONS OF PYTHIUM MYRIOTYLUM WITH FUSARIUM SOLANI, RHIZOCTONIA SOLANI, AND OTHER FUNGI, AND WITH MELOIDOGYNE ARENARIA IN PEANUT POD ROT AND PREEMERGENCE DAMPING-OFF

By

Roberto Garcia

December, 1974

Chairman: David J. Mitchell
Major Department: Plant Pathology

Interactions of Pythium myriotylum with several fungi and with Meloidogyne arenaria in preemergence damping-off and pod rot of peanut were investigated by exposing seedlings or pods to defined inoculum densities of the various fungi or the nematode. Autoclaved soil was directly infested with spores or resting structures of the fungi, or was artificially infested with the fungi, assayed for the populations of fungi, and diluted to contain desired levels of each fungus. Nematodes were added directly as eggs in all experiments.

A synergistic interaction between P. myriotylum and Fusarium solani occurred in damping-off of seedlings as well as in pod rot. Synergism was also observed between P. myriotylum and M. arenaria with seedlings exposed to directly infested soil and with pods exposed to infested-diluted soil.

The following combinations of organisms also acted synergistically in damping-off of seedlings: Rhizoctonia solani plus Trichoderma viride, R. solani plus Macrophomina phaseolina, R. solani plus M. arenaria, and T. viride plus M. arenaria.

The exposure of attached or detached pods to R. solani prevented or reduced the development of pod rot in pods later exposed to P. myriotylum in soil or in vitro. High populations of M. phaseolina nullified the antagonistic effect of R. solani on P. myriotylum if the pods were grown in soil, but not if the pods were grown in vermiculite.

The isolation of P. myriotylum from pods or roots was reduced after exposure to soil containing R. solani. This effect was most striking with attached or detached pods which were successively exposed to R. solani in soil or in vitro prior to exposure to P. myriotylum in soil. Rhizoctonia solani, on the other hand, was isolated much less frequently or could not be isolated from attached or detached pods exposed to P. myriotylum prior to exposure to R. solani. If pods were exposed to F. solani or T. viride in vitro prior to R. solani and then P. myriotylum in soil, pod rot occurred but no P. myriotylum and little or no R. solani were isolated from the pods. However, when pods were exposed to R. solani in vitro prior to F. solani or T. viride and then P. myriotylum in soil, pod rot was not greater than in the control and R. solani but not P. myriotylum could be

isolated from the pods. The presence of R. solani in the soil with peanut seedlings also greatly reduced or eliminated recovery of P. myriotylum from soil.

PART 1

INTERACTIONS OF *PYTHIUM MYRIOTYLUM* WITH *FUSARIUM SOLANI*, AND *MELOIDOGYNE ARENARIA* ON PEANUT SEEDLINGS

Introduction

Although most of the pathogenicity studies with *Pythium myriotylum* Drechs. on peanut (Arachis hypogaea L.) have been concerned with its role in pod rot, it has been reported to also cause seed decay, and pre- and post-emergence damping-off, root rot, and vascular wilt (1, 13, 17, 34). It is also known to be pathogenic on several grass and vegetable crops (14, 25).

Epiphytotics of *Pythium* wilt, caused mainly by *P. myriotylum*, have been reported occasionally from Virginia, but the disease is normally limited to small groups of plants scattered throughout fields (34). Bell and Minton (1) reported up to 74% post-emergence damping-off of the Spanish type cultivar 'Star' after inoculation with *P. myriotylum*. Wills and Moore (42) observed up to 100% of young seedlings diseased after inoculation with *P. myriotylum*.

Pythium myriotylum was observed to be associated with *Meloidogyne arenaria* (Neal) Chitwood in severely galled and rotted roots and pods of peanuts in a field in Levy County, Florida in 1972 (Mitchell, D. J.,personal communication). Although *Meloidogyne* spp. have been reported to cause severe

injury to root systems of Spanish type peanuts (38) and cause economic losses in many instances (13), little evidence is available on the interactions of Meloidogyne spp. and fungal pathogens in peanuts. Minton (30) suggested that the necroses caused by the less virulent of two populations of M. arenaria could possibly provide points of attack for other organisms resulting in a disease complex. Minton and Jackson (31,32) found that the presence of M. arenaria resulted in an increase in numbers of total fungi in shells, but that there was not a consistent increase in the invasion of pods by Aspergillus flavus (Link) Fries in the presence of M. arenaria. Bell et al. (2) concluded in a later report that M. arenaria damage to peanut pods did not affect A. flavus infection.

Root-knot nematodes and various species of Pythium are known to interact in diseases of other hosts (36). Johnson and Littrell (18, 23) showed that the presence of M. incognita (Kofoid and White) Chitwood increased damage by P. aphanidermatum (Edson) Fitzp. on chrysanthemum and that egg production of M. incognita was suppressed in plants infected with the fungus. Melendez and Powell (27, 28) found that only slight root decay of root-knot susceptible tobacco plants occurred when plants were inoculated simultaneously with both M. incognita and P. ultimum Trow ; severe decay, however, occurred when plants were inoculated with root-knot nematodes 4 weeks prior to the fungus inoculation. A reduction in the nematode population was observed in the root systems to which the fungus was added 2 weeks before the nematodes, but the nematode pop-

ulation increased with time when M. incognita preceded the fungus.

Although no reports on interactions of M. arenaria and P. myriotylum or of P. myriotylum and other fungal pathogens on peanut seedlings were found in a literature review, interactions are known to occur with P. myriotylum and various other organisms in peanut pod-rot. Frank (7) concluded that P. myriotylum and F. solani (Mart.) App. & Wr. emend Snyd. & Hans interact synergistically since neither pathogen alone was an effective pathogen. The inoculation of pods in autoclaved soil with F. solani followed by inoculation with P. myriotylum after 4 wk yielded a significantly higher proportion of diseased pods than did inoculation with P. myriotylum followed by inoculation with F. solani. Several fungi other than F. solani have been found to be associated with P. myriotylum in pod rot of peanut (8, 13, 15, 17, 22). Beaute (3) observed that interactions between mites and various fungi in pod rot were indicated by partial control of pod rot after the application of acaricides to eliminate mites from field soil. Porter and Smith (35) observed that pod rot caused by P. myriotylum was greatly enhanced by the presence of southern corn rootworm larvae.

The objectives of this study were to evaluate the interactions of various fungal-fungal and nematode-fungal combinations at defined inoculum densities of P. myriotylum, F.

solani, Rhizoctonia solani Kuhn, Macrophomina phaseolina (Tassi) Goid, Trichoderma viride Pears. ex Fries, and M. arenaria on root-rot and damping-off of peanut seedlings.

Materials and Methods

The fungi used in this study were isolated from rotted peanut pods. Hyphal tipped isolates of Rhizoctonia solani, Pythium myriotylum, Macrophomina phaseolina, Fusarium solani, and Trichoderma viride were maintained on V-8 juice agar (200 ml Campbell's V-8 juice, 4.5 g $CaCO_3$, 17 g Difco agar, and 800 ml distilled water).

Eggs of Meloidogyne arenaria were extracted from galled peanut roots and the nematode maintained on tomato, Lycopersicon esculentum Mill., 'Bonnie Best'.

Inoculum was prepared for each fungus by adding a 4-mm disk from the margin of a 48-hr-old V-8 juice agar culture to a 250-ml Erlenmeyer flask containing 50 ml of half strength V-8 juice broth (100 ml Campbell's V-8 juice, 4.5 g of $CaCO_3$, and 800 ml of distilled water). The inoculated flasks were maintained at room temperature (27-30 C) under continuous light for 15 days.

Arredondo fine sand with a pH of 6.5 (measurement obtained from a 1:2 suspension of soil in 0.01 M $CaCl_2$) was used throughout this study after it was autoclaved twice for 4 h at 24 h intervals at 15 psi and 120 C.

The fungi and nematodes were mixed with the soil with an electric hand mixer. Water was added to the soil during the mixing process to give a final water content of 10% (w/w).

Soil was infested for the first experiment by Aceptically mixing eight cultures of each fungus into 1 liter of autoclaved soil. After the infested soil had been maintained at approximately 27 C for 15 days in 2-liter Mason jars, 15 g samples of soil were removed from each jar for population evaluation of the fungal pathogens.

Populations of P. myriotylum were evaluated by preparing a soil dilution of 1:25 (w/v) in 0.25% water agar containing 300 mg/liter vancomycin hydrochloride (Vancocin, Eli Lilly & Co.) and 3.68 g/liter of $CaCl_2.2H_2O$. A 1-ml aliquot was spread with a sterile glass rod over each of 10 Petri plates containing a selective medium for pythiaceous fungi modified from that of Tsao and Ocana (39). This medium consisted of 17 g of corn meal agar (Difco) plus 5 mg of pimaricin (Delvocid, Gist Brocades, Delfet, Holland) and 300 mg of vancomycin in 1 liter of distilled water (PV). After 36 h of incubation in the dark at 30 C, the soil agar suspension was washed from the surface of the plates under a slow stream of tap water and the colonies of P. myriotylum were counted.

Populations of R. solani in infested soil were evaluated with the selective medium (GSC) described by Ko and Hora (20). Ten grams of soil were mixed on a 5 x 10 x 0.5 cm tray with 10 ml of 2.5% water agar containing 50 mg/liter of streptomycin sulfate (Eli Lilly & Co.). The solidified agar-soil sample

was divided into 100 squares and 10 squares were evenly distributed on each of 10 GSC plates. After incubation in the dark at 30 C for 36 h, the colonies of R. solani were counted.

Populations of M. phaseolina in infested soil were estimated by using a selective medium (CMR) described by Meyer et al. (29). One gram of soil was suspended in 100 ml of sterile tap water ammended with 50 mg/liter of streptomycin sulfate, and 1-ml aliquot was spread over each of 10 CMR plates. After incubation in the dark at 30 C for 7 days the M. phaseolina colonies formed dark sclerotia and could be easily counted.

For the population evaluation of F. solani and T. viride, one gram of soil was suspended in one liter of sterile tap water. A 1-ml aliquot was placed on the bottom of each 10 sterile Petri plates and mixed with 15 ml of molten medium (PDATS) described by Steiner and Watson (37). The PDATS consisted of potato dextrose agar, 1 ml/liter of tergitol NPX and 50 mg/liter of streptomycin sulfate. After incubation at approximately 27 C under continuous fluorescent light for 5 to 7 days, the colonies of F. solani or T. virdie were counted.

Meloidogyne arenaria eggs were extracted by the method of McClure et al. (26) from the roots of tomato plants. The number of eggs per ml of a suspension of eggs was evaluated by counting the number of eggs in a 210 mm^2 area for 12 samples.

After the population of each fungus was determined, the infested soil was diluted with sterile soil to establish the

desired inoculum density for each pathogen. Known numbers of eggs of M. arenaria were added to soil to establish nematode populations.

The first experiment was conducted to evaluate the influence of the various pathogens singly or in all possible combinations of pairs of pathogens at the following inoculum densities on peanut seedling disease: P. myriotylum, 80 propagules/gram of soil (ppg); F. solani, 3,000 ppg; R. solani, 0.8 ppg; M. phaseolina, 200 ppg; T. viride 30,000 ppg; and M. arenaria, 5 eggs/ml of soil.

Inoculum densities of the fungal pathogens in soil for the second experiment were prepared by adding specific numbers of spores or resting structures to soil. Oospores of P. myriotylum were obtained by washing 5 matts of the fungus from V-8 juice broth cultures with sterile distilled water and blending the cultures in 50 ml of sterile distilled water for 20 sec at maximum intensity in a microblender. The resulting suspension was subjected to 60% maximum sonification with a Biosonik III ultrasonic system for 40 sec to leave only oospores as viable propagules in the suspension. The number of oospores in the suspension was determined by counting six fields for each of 12 samples in a standard haemocytometer.

Mycelial mats of F. solani were blended in their own culture media for 45 sec at maximum intensity in a microblender. The suspension was passed through a 45 µm sieve and centrifuged at 1000 g for 5 minutes. The pellet

was resuspended in sterile distilled water and the number of conidia per ml was evaluated by counting six fields for each of 12 samples in a standard haemocytometer.

Mycelial mats of R. solani were blended in sterile tap water for 45 sec at maximum intensity in a blender, and the resulting suspension was passed through nested 125 and 250 µm sieves. The sclerotia were retained and the mycelia fragments were removed by exposure to a high pressure water spray. After resuspending the sclerotia in tap water, the number of sclerotia/ml was evaluated by counting the sclerotia in twelve 210 mm^2 samples.

Eggs of M. arenaria were extracted and calculated as described for the first experiment.

The second experiment was conducted to evaluate the influence of the following pathogens, and combination of pathogens at the indicated inoculum densities on peanut seedling disease: P. myriotylum, 200 oospores/gram of soil; F. solani, 1000 conidia/gram of soil; R. solani, 10 sclerotia/gram of soil; and M. arenaria, 5 eggs/ml of soil. The combinations were P. myriotylum plus F. solani, P. myriotylum plus M. arenaria, P. myriotylum plus R. solani, R. solani plus M. arenaria, and F. solani plus M. arenaria, and they had the same inoculum density indicated for each pathogen by itself.

All experiments were conducted in autoclaved, 15-cm clay pots in a greenhouse. Ten pots and 21 pots per treatment for the first and second experiment, respectively, were prepared by placing a layer of 120 g of infested soil on top

of 180 g of autoclaved soil, and covering the infested soil with 120 g of autoclaved soil. A germinated Florunner peanut seed was placed in each pot and covered with 60 g of sterile soil. Seeds were germinated in sterile vermiculite in Petri dishes after they had been surface disinfested with 1.0% by weight sodium hypochlorite for 40 sec and rinsed three times with sterile water.

Two groups of five plants and three groups of seven plants per treatment were randomized for the first and second experiments, respectively.

The plants were harvested after 21 days and results were analyzed by evaluating the percentage of preemergence damping-off, number of leaves, width of largest leaf per plant and height and volume (by water displacement) of the plants. The root systems of plants exposed to P. myriotylum, and R. solani were plated on PV and GSC media, respectively, after surface sterilization with 1.0% sodium hypochlorite for 30 seconds. The root systems of plants exposed to pathogens other than P. myriotylum or R. solani were plated on lactic acid acidified (pH 4) PDA (APDA).

Soil samples were taken before the plants were removed from the infested soil layer and the populations of P. myriotylum were evaluated with PV, R. solani with GSC, and F. solani with PDATS.

Because the root systems were always plated for fungal infection evaluation, no precise evaluation was performed for M. arenaria other than the determination of the presence of young galls and the presence of young females in roots.

Results

Only the combination of P. myriotylum and F. solani resulted consistently in a significantly greater percentage of damping-off of peanut seedlings than the sum of effects of the two separate pathogens with both types of soil infestation (Tables 1,2). This synergistic interaction was observed with artifically infested soil diluted with autoclaved soil to contain P. myriotylum at 80 ppg of soil and F. solani at 3000 ppg of soil (Table 1) and with autoclaved soil directly infested with 200 oospores of P. myriotylum/g of soil and 1000 conidia of F. solani/g of soil (Table 2). When the influence of the various treatments on peanut seedlings were evaluated by observing the number of leaves per plant, widths of largest leaf, plant height, or plant volume synergistic interaction was indicated between P. myriotylum and F. solani by differences in plant volume with the soil dilution method and by widths of the largest leaf with both soil infestation methods (Tables 3,4).

Synergism also occurred between P. myriotylum and M. arenaria when soil was infested directly with 200 oospores of P. myriotylum per gram of soil and 5 eggs of M. arenaria/ml of soil (Table 2). The difference, however, was not significant between P. myriotylum alone and P. myriotylum in combination with M. arenaria in soil infested and diluted with autoclaved soil to contain P. myriotylum at 80 ppg of soil (Table 2). Of the parameters other than damping-off, only the width of the largest leaf of plants grown in directly infested soil

indicated synergism between P. myriotylum and M. arenaria (Tables 3,4).

Although neither M. arenaria nor Trichoderma viride alone caused damping-off of seedlings, a synergistic interaction was observed between the two organisms with seedlings grown in soil infested by the dilution method (Table 1). Synergism was not indicated by measurements of any of the other parameters (Table 3). Synergistic interactions were also observed with R. solani plus M. arenaria or T. viride in infested-diluted soil.

Although the percentage of damping-off in directly infested soil was greater than the control only with combinations of P. myriotylum plus F. solani or M. arenaria, (Table 2), significantly (0.05) higher percentages of damping-off occurred with several pathogens when soil was infested by the dilution method (Table 1). All treatments containing P. myriotylum, except when it was combined with M. phaseolina, resulted in 40% or more damping-off. Significant percentages of damping-off also occurred in soil containing R. solani, F. solani, M. phaseolina, or combinations of F. solani plus M. phaseolina, T. viride plus M. arenaria, R. solani plus M. phaseolina, R. solani plus T. viride, and R. solani plus M. arenaria.

Plant height was significantly reduced over that of the control in directly infested soil by interactions between P. myriotylum plus F. solani and P. myriotylum plus M. arenaria (Table 4). Pythium myriotylum alone or in combination with

M. arenaria, R. solani or F. solani significantly reduced the height of plants exposed to soil prepared by dilution of infested soil; plant heights were also reduced when exposed to combinations of R. solani plus M. arenaria, T. viride or F. solani (Table 3).

The number of leaves per plant was significantly reduced compared with the control with P. myriotylum alone or in combinations with F. solani or M. arenaria in directly infested soil (Table 4), and with P. myriotylum alone or in combinations with F. solani, R. solani or M. arenaria in infested-diluted soil (Table 3).

The number of leaves per plant was also significantly reduced in infested-diluted soil combinations containing F. solani plus T. viride, F. solani plus M. phaseolina, R. solani plus M. arenaria, R. solani plus T. viride, and R. solani plus F. solani (Table 3).

Plant volume was reduced significantly when compared with the control in all treatments except R. solani, M. arenaria and R. solani plus M. arenaria in the directly infested soil (Table 4). In infested-diluted soil, the plant volume was reduced significantly over the control by all treatments except R. solani, F. solani plus M. arenaria, M. phaseolina plus M. arenaria, and T. viride plus M. arenaria treatments in the infested-diluted soil (Table 3).

In the infested-diluted soil treatments containing P. myriotylum, the retrieval of this fungus from roots varied from 10% for P. myriotylum by itself to 40% for P. myriotylum

plus T. viride but the differences were not significant (Table 5).

Pythium myriotylum was isolated from the root systems of 19-33% of the peanut seedlings exposed to it in the directly infested soil experiments, except when it was combined with R. solani (Table 6).

Little differences were observed in the retrieval of R. solani within treatments exposed to it in the directly infested soil (Table 6), and it was not recovered at all from the infested-diluted soil (Table 5).

Fusarium solani was isolated even from roots in treatments that did not include it. The frequency of recovery, however, was significantly higher in those treatments to which F. solani had been added by direct soil inoculation (Table 6).

The population of P. myriotylum was lower in the presence of R. solani in soil infested by both methods (Table 7). The highest P. myriotylum populations were observed in the infested soil that had been diluted to contain 80 ppg of P. myriotylum, especially when P. myriotylum was combined with T. viride. In soil that had been directly infested with 200 oospores of P. myriotylum/g of soil, the highest population recovered was only 10 ppg when P. myriotylum was combined with M. arenaria.

Populations of F. solani and R. solani were higher in directly inoculated soil than in infested-diluted soil (Table 7). The population of F. solani was reduced in soil containing R. solani.

Table 1. Percentage of preemergence damping-off of Florunner peanut seedlings after exposure for 3 wk to various pathogens alone or in combinations at defined inoculum densities[y] in infested soil diluted with sterile soil.

Treatment	Damping-off (%)
Non-inoculated	0.0 a[z]
P. myriotylum + *M. phaseolina*	0.0 a
M. arenaria	0.0 a
T. viride	0.0 a
R. solani + *F. solani*	10.0 ab
F. solani + *T. viride*	10.0 ab
M. phaseolina + *T. viride*	10.0 ab
M. phaseolina + *M. arenaria*	10.0 ab
R. solani	20.0 bc
F. solani	20.0 bc
M. phaseolina	20.0 bc
F. solani + *M. arenaria*	20.0 bc
F. solani + *M. phaseolina*	30.0 bc
T. viride + *M. arenaria*	30.0 bc
P. myriotylum	40.0 c
P. myriotylum + *R. solani*	40.0 c
P. myriotylum + *T. viride*	40.0 c
P. myriotylum + *M. arenaria*	40.0 c
R. solani + *M. phaseolina*	40.0 c
R. solani + *T. viride*	40.0 c
R. solani + *M. arenaria*	50.0 c
P. myriotylum + *F. solani*	90.0 d

[y]Inoculum densities were: *Pythium myriotylum*, 80 propagules per gram of soil (ppg); *Rhizoctonia solani*, 0.8 ppg; *Macrophomina phaseolina*, 200 ppg; *Fusarium solani*, 3,000 ppg; *Trichoderma viride*, 30,000 ppg; and *Meloidogyne arenaria*, 5 eggs/ml of soil.

[z]Values (presented as means of two replicates of 5 plants each) followed by the same letter are not significantly different (P= 0.05 by Duncan's Multiple Range Test). Analysis performed with data transformed to arcsin degrees.

Table 2. Percentage of preemergence damping-off of Florunner peanut seedlings exposed for 3 wk to different pathogens alone or in combinations in soil to which known quantities of reproductive structures were added[y].

Treatment	Damping-off (%)
Non-inoculated	0.00 a[z]
M. arenaria	0.00 a
R. solani	0.00 a
R. solani + *M. arenaria*	0.00 a
P. myriotylum	4.73 a
F. solani	4.73 a
P. myriotylum + *R. solani*	4.73 a
F. solani + *M. arenaria*	4.73 a
P. myriotylum + *F. solani*	28.50 b
P. myriotylum + *M. arenaria*	33.28 b

[y]Inoculum densities were: *Pythium myriotylum*, 200 oospores per gram of soil; *Rhizoctonia solani*, 10 sclerotia/g of soil; *Fusarium solani*, 1,000 conidia/g of soil; and *Meloidogyne arenaria*, 5 eggs/ml of soil.

[z]Values followed by the same letter are not significantly different (P=0.05 by Duncan's Multiple Range Test); Analysis performed with data transformed to arcsin degrees (21 plants/repetition).

Table 3. Height, width of largest leaf, and plant volume of Florunner peanut seedlings exposed for 3 wk to various pathogens alone or in combinations at defined inoculum densities[y] in infested soil diluted with sterile soil.

Treatment	Plant height (cm)	Width of largest leaf (mm)	No. of leaves	Plant volume (ml)
Non-inoculated	11.30 a[z]	15.90 ab	31.20 a	7.60 a
F. solani + M. arenaria	12.70 a	16.70 a	30.60 a	8.10 ab
F. solani	12.20 a	17.60 a	25.20 a	7.00 a
M. phaseolina + M. arenaria	11.50 a	15.00 ab	25.60 a	7.00 a
R. solani	11.18 a	17.08 a	23.60 a	4.54 b
M. phaseolina + T. viride	10.40 ab	14.50 ab	20.80 a	5.50 b
R. solani + M. phaseolina	10.40 ab	14.60 ab	21.60 ab	5.70 b
T. viride	9.80 ab	13.60 ab	25.60 a	5.40 b
M. arenaria	9.50 ab	16.10 ab	22.80 ab	5.40 b
M. phaseolina	9.50 ab	13.30 ab	23.20 ab	5.00 b
T. viride + M. arenaria	8.80 ab	11.90 ab	18.40 ab	8.70 a
P. myriotylum + M. phaseolina	8.20 ab	14.00 ab	20.00 ab	4.30 b
F. solani + T. viride	8.00 ab	13.60 ab	17.20 b	5.30 b
P. myriotylum + T. viride	8.00 ab	11.20 ab	16.00 b	3.70 bc
F. solani + M. phaseolina	7.40 ab	10.70 ab	17.60 b	4.50 b
P. myriotylum + M. arenaria	6.70 b	11.50 ab	15.20 b	4.20 b
R. solani + M. arenaria	6.60 b	8.70 b	13.20 bc	5.00 b
P. myriotylum + R. solani	6.30 b	10.40 ab	14.40 b	4.30 b
P. myriotylum	5.70 bc	10.20 ab	13.20 bc	4.70 b
R. solani + T. viride	5.20 bc	8.70 b	9.60 bc	3.30 bc
R. solani + F. solani	5.20 bc	9.20 b	10.40 bc	3.70 bc
P. myriotylum + F. solani	0.60 c	1.60 c	1.60 c	1.60 c

[y]Inoculum densities were: Pythium myriotylum, 80 propagules per gram of soil (ppg); Rhizoctonia solani, 0.8 ppg; Macrophomina phaseolina, 200 ppg; Fusarium solani, 3000 ppg; Trichoderma viride, 30,000 ppg; and Meloidogyne arenaria, 5 eggs/ml of soil.

[z]Values (means of ten plants) in a vertical column followed by the same letter are not significantly different (P=0.05 by Duncan's Multiple Range Test).

Table 4. Height, width of largest leaf, number of leaves, and plant volume of Florunner peanut seedlings exposed to various pathogens alone or in combinations in soil to which known quantities of reproductive structures were added.[y]

Treatment	Plant height (cm)	Width of largest leaf (mm)	No. of leaves	Plant volume (ml)
Non-inoculated	14.90 a[z]	21.23 a	46.00 a	13.47 a
R. solani	14.19 a	20.61 a	45.52 a	11.14 ab
F. solani	14.33 a	19.90 a	42.38 a	9.66 bc
M. arenaria	13.28 a	19.47 a	41.38 ab	10.90 ab
R. solani + *M. arenaria*	13.04 a	19.61 a	40.42 ab	11.33 a
P. myriotylum + *R. solani*	13.38 ab	19.47 a	40.33 ab	8.47 bc
F. solani + *M. arenaria*	13.95 ab	19.90 a	39.23 ab	9.28 bc
P. myriotylum	12.09 ab	17.80 a	33.61 bc	8.04 bc
P. myriotylum + *F. solani*	10.14 b	12.95 b	30.47 c	7.66 c
P. myriotylum + *M. arenaria*	9.04 b	12.76 b	27.33 c	7.80 bc

[y]Inoculum densities were: *Pythium myriotylum*, 200 oospores/g of soil; *Rhizoctonia solani*, 10 sclerotia/g of soil; *Fusarium solani*, 1000 conidia/g of soil; and *Meloidogyne arenaria*, 5 eggs/ml of soil.

[z]Values (means of 21 plants) in a vertical column followed by the same letter are not significantly different (P=0.05 by Duncan's Multiple Range Test).

Table 5. Recovery of Pythium myriotylum, Rhizoctonia solani, and Fusarium solani from the root system of Florunner peanut plants exposed for 3 weeks to inoculum densities[x] prepared by diluting infested soil with autoclaved soil.

Treatments	Frequency of isolation (%)[y]		
	P. myriotylum	R. solani	F. solani
P. myriotylum	10.0 a[z]	---	0.0
R. solani	-----	0.0	0.0
P. myriotylum + R. solani	30.0 a	0.0	0.0
P. myriotylum + M. phaseolina	30.0 a	---	0.0
P. myriotylum + F. solani	30.0 a	---	0.0
P. myriotylum + T. viride	40.0 a	---	0.0
P. myriotylum + M. arenaria	20.0 a	---	0.0
R. solani + M. phaseolina	-----	0.0	0.0
R. solani + F. solani	-----	0.0	0.0
R. solani + T. viride	-----	0.0	0.0
R. solani + M. arenaria	-----	0.0	0.0

[x]Inoculum densities were: P. myriotylum, 80 propagules per gram of soil; R. solani, 0.8 ppg; F. solani, 3000 ppg; Macrophomina phaseolina, 200 ppg; Trichoderma viride, 30,000 ppg; and Meloidogyne arenaria, 5 eggs/ml of soil.

[y]All roots were heavily covered with T. viride.

[z]Recovery from treatments within any given pair in a vertical column followed by the same letter is not different at the 0.05 level of significance, compared with the contingency chi square test.

Table 6. Recovery of Pythium myriotylum, Rhizoctonia solani, and Fusarium solani from the root systems of Florunner peanut plants exposed for 2 weeks to soil infested with known quantities[y] of reproductive structures.

Treatments	Frequency of isolation (%)					
	P. myriotylum		R. solani		F. solani	
	No.[x]	%	No.	%	No.	%
P. myriotylum	5 b[z]	23.80	---	---	4 a	19.04
F. solani	---	---	---	---	21 b	100.00
M. arenaria	---	---	---	---	9 a	42.85
R. solani	---	---	12 b	57.14	4 a	19.04
P. myriotylum + F. solani	4 b	19.04	---	---	21 b	100.00
P. myriotylum + M. arenaria	7 b	33.33	---	---	2 a	9.52
P. myriotylum + R. solani	0 a	0.0	13 b	61.90	2 a	9.52
F. solani + M. arenaria	---	---	---	---	21 b	100.00
Non-inoculated	0 a	0.0	0 a	0.0	5 a	23.80
R. solani + M. arenaria	---	---	11 b	52.38	3 a	14.28

[x]Twenty-one plants used for each treatment.

[y]Inoculum densities were: P. myriotylum, 200 oospores per gram of soil; R. solani, 10 sclerotia/g of soil; F. solani, 1000 conidia/g of soil; and Meloidogyne arenaria, 5 eggs/ml of soil.

[z]Retrieval from treatments within any given pair in a vertical column followed by the same letter is not different at the 0.05 level of significance, compared with the contingency chi square test.

Table 7. Recovery of Pythium myriotylum, Rhizoctonia solani, and Fusarium solani from soil after 3 weeks of exposure of Florunner peanut seedlings to defined inoculum densities prepared by diluting infested soil with autoclaved soil[x] or by directly infesting soil with known numbers of reproductive structures[y].

Treatment	Recovery (propagules/g of soil)					
	Infested-diluted soil			Directly infested		
	P. myriotylum	R. solani	F. solani	P. myriotylum	R. solani	F. solani
Non-inoculated	0.0	0.0	0	0.0	0.0	0
P. myriotylum	32.0	---	---	5.0	---	---
P. myriotylum + R. solani	4.0	0.5	---	0.0	5.2	---
P. myriotylum + F. solani	48.0	---	12700	5.0	---	15500
P. myriotylum + T. viride	88.0	---	---	---	---	---
P. myriotylum + M. phaseolina	44.0	---	---	---	---	---
P. myriotylum + M. arenaria	28.0	---	---	10.0	---	---
F. solani	--- [z]	---	13900	---	---	32300
R. solani + F. solani	---	0.2	0	---	---	---
F. solani + M. phaseolina	---	---	17900	---	---	---
F. solani + T. viride	---	---	2500	---	---	---
F. solani + M. arenaria	---	---	13800	---	---	30100
R. solani	---	1.2	---	---	4.4	---
R. solani + M. arenaria	---	0.0	---	---	10.2	---
R. solani + M. phaseolina	---	1.1	---	---	---	---
R. solani + T. viride	---	0.2	---	---	---	---

[x]Infested soil diluted with autoclaved soil to give 80 propagules per gram of soil (ppg) of P. myriotylum, 0.8 ppg of R. solani, and 3000 ppg of F. solani.

[y]Soil infested by adding 200 oospores of P. myriotylum/g of soil, 10 sclerotia of R. solani/g of soil, and 1000 conidia of F. solani/g of soil.

[z]--- = Not sampled.

Discussion

The synergistic interactions between P. myriotylum and F. solani, and between P. myriotylum and M. arenaria on damping-off of peanut seedlings were affected strongly by the inoculum densities of P. myriotylum. When soil with an initial inoculum density of 200 oospores/g of soil was sampled after 21 days, it had lower levels of P. myriotylum than infested soil that had been diluted with autoclaved soil to contain an assayed initial level of 80 ppg. Either only a low proportion of the oospores were viable, or the recovery of P. myriotylum from soil is very low. High levels of damping-off occurred with corresponding combinations of P. myriotylum and other fungi in soil infested at the higher inoculum density by the dilution of infested soil with autoclaved soil than in soil infested at the lower inoculum density by addition of oospores.

Although combinations of P. myriotylum with F. solani or M. arenaria in directly infested soil resulted in synergistic interactions, none of the organisms alone caused significant amounts of damping-off. Frank (7) may also have been dealing with low inoculum densities when he observed a synergistic interaction between P. myriotylum and F. solani in pod rot of peanut and noted that neither fungus alone was an effective pathogen. Since F. solani has been commonly isolated from peanut pods grown in treated or non-treated soil (7, 11) it probably plays an important, but often unrecognized, role in peanut pod rot when P. myriotylum is present.

With the higher inoculum density of P. myriotylum obtained with the dilution of infested soil, P. myriotylum and F. solani also interacted synergistically but each fungus alone caused damping-off as well. Kraft (21) reported a similar effect after observing that P. ultimum and F. solani f. sp. pisi, each at approximately 1,000 ppg, were individually capable of causing diseases in peas, but that pea root rot was more severe when both pathogens were present. This effect also has been observed with several other Pythium spp. in combination with F. solani f. sp. pisi and F. oxysporum f. sp. pisi (5, 19).

In contrast with the synergistic interaction observed between P. myriotylum and M. arenaria when the former is present at low inoculum densities in directly infested soil, the addition of M. arenaria to soil containing the high inoculum density of P. myriotylum in infested-diluted soil did not significantly increase damping-off in comparison to P. myriotylum alone. The importance of defining the inoculum density of root rotting fungi and nematodes when evaluating interactions has also been demonstrated by Oyekan and Mitchell (33). They observed that the presence of the root lesion nematode, Pratylenchus penetrans had little effect on root rot of canning pea when the inoculum density of Aphanomyces euteiches was high, but significant increases in root rot were observed in the presence of the nematode at lower inoculum densities of the fungus.

Synergistic interactions in damping-off of seedlings were also observed between combinations of T. viride, R. solani, and M. arenaria in infested-diluted soil but these combinations should be reevaluated under carefully controlled environmental conditions and inoculum densities with greater number of plants. The presence of T. viride in soil apparently strongly influences the recovery of other fungi from roots. Neither R. solani nor F. solani, for example, could be isolated from roots that were heavily infested with T. viride (Table 5). Trichoderma viride, however, has been reported to interact with Pratylenchus penetrans in alfalfa and celery (4) and should be considered a possible cofactor in disease complexes.

When M. phaseolina was combined with P. myriotylum in infested-diluted soil, no damping-off occurred (Table 1). This antagonistic effect in P. myriotylum has been observed in pod rot of peanut with R. solani but not M. phaseolina (Part 2 of this dissertation). The antagonistic effect of R. solani on Pythium aphanidermatum in soil has been reported (6, 24). In this study the presence of R. solani, but not the presence of M. phaseolina, reduced P. myriotylum populations in soil (Table 7).

It has been demonstrated in this study that P. myriotylum is capable of causing damping-off of seedlings when it is present at high inoculum densities under favorable environmental conditions. More severe damping-off occurs when P. myriotylum is combined with F. solani or M. arenaria. The

influence of P. myriotylum has also been shown to be much greater in peanut pods simultaneously exposed to other organisms such as other fungi (7), soil mites (3), or southern corn rootworm (35).

PART 2

INTERACTIONS OF *PYTHIUM MYRIOTYLUM* WITH SEVERAL FUNGI AND *MELOIDOGYNE ARENARIA* IN PEANUT POD ROT

Introduction

Many plant pathogenic fungi and nematodes have been associated with peanut (Arachis hypogaea L.) pod rot. As of 1973, a total of 110 genera and approximately 200 species of fungi have been isolated from peanuts (8, 13, 15). Species in nine genera of plant parasitic nematodes have been reported to cause injury to peanuts (13).

Interactions among some of the fungi isolated from pods have been reported (9, 10, 16, 41). *Trichoderma viride* Pears. ex Fries reduced colonization of immature and mature pericarps by *Aspergillus flavus* (Link.) Fr., but this antagonistic effect was nullified in the presence of *Penicillium funiculosum* Thom which also stimulated colonization of mature pericarps and testae by *A. flavus* (41). *Aspergillus flavus* has been observed to reduce the growth and spread of *Macrophomina phaseolina* (Tassi) Goid, when pods and kernels were inoculated simultaneously with both fungi (16). Garren (10) considered *Pythium myriotylum* Drechs. and *Rhizoctonia solani* Kuhn to be capable of causing peanut pod breakdown (pod rot), and he suggested that the competition between the two fungi resulted in domination by

P. myriotylum over R. solani in causing pod rot under most conditions. Garren (9), however, in earlier work pointed out that the greater competitive ability of T. viride over R. solani in decaying pods makes it difficult to assess the role of R. solani in the pod rot disease. Frank (7) found that P. myriotylum and Fusarium solani (Mart.) App. & Wr. emend Snyd. & Hans interact synergistically in peanut pod rot. He considered that F. solani not only predisposes the pods to attack by P. myriotylum but that it is also involved in the final disintegration of diseased pods.

Other organisms also interact with fungal pathogens in peanut pod rot (3, 35). Minton and Jackson (31, 32) found that the presence of Meloidogyne arenaria (Neal)Chitwood resulted in an increase in numbers of total fungi in shells, but the increase in the invasion of pods by A. flavus in the presence of M. arenaria was not consistent. Beute (3) observed that interactions between mites and various fungi in pod rot were indicated by partial control of pod rot after the application of acaricides to eliminate mites from field soil. Porter and Smith (35) observed that pod rot caused by P. myriotylum was greatly enhanced by the presence of southern corn rootworm larvae.

Pythium myriotylum, R. solani, F. solani, M. phaseolina, and T. viride were consistently isolated from sound and rotted peanut fruits from plants grown in a farm with a high incidence of pod rot in 1972 in Levy County, Florida. Meloidogyne arenaria was also observed to cause severe galling

of pods in the same field (Mitchell, D. J., personal communication). Combinations of P. myriotylum plus F. solani and P. myriotylum plus M. arenaria were observed to interact synergistically in preemergence damping-off of peanut seedlings in the greenhouse (see part I of this dissertation). The objectives of this study were to determine the influence of various fungal-fungal or fungal-nematode combinations at defined or mass inoculum densities on peanut pod rot.

Materials and Methods

Inoculum production. The fungi used throughout this investigation were isolated from rotted peanut pods obtained from a field located in township 13 S, range 18 E, section 3 of Levy County, Florida. Hyphal-tipped cultures of Fusarium solani, Macrophomina phaseolina, Pythium myriotylum, Rhizoctonia solani, and Trichoderma viride were maintained on V-8 juice agar (200 ml Campbell's V-8 juice, 4.5 g $CaCO_3$, 17 g Difco agar, and 800 ml distilled water).

Fungal inoculum for soil infestation was prepared by adding a 4-mm disk from the margin of a 48-h-old V-8 juice agar culture of each fungus to a 250 ml Erlenmeyer flask containing 50 ml of V-8 juice broth (100 ml Campbell's V-8 juice, 4.5 g $CaCO_3$, and 800 ml distilled water). The flasks were maintained at approximately 25 C under continuous light for 15 days.

Eggs of Meloidogyne arenaria were extracted from galled peanut roots and populations of the nematode were maintained on tomato , Lycopersicon esculentum Mill, 'Bonnie Best'.

Peanut pod production. Florunner peanut plants were grown in the greenhouse at 27 to 35 C. Peanut seeds were soaked in running tap water for 24 hr, surface disinfested with 1.0% sodium hypochlorite for 40 seconds, rinsed three times with sterile tap water, and placed to germinate in moist, sterile vermiculite in Petri dishes. One germinated seed was placed in the top of an open-ended tube (25-cm long and 5-cm diam) filled with soil. Each tube was placed on top of soil contained in a 20-liter can filled to 0.75 of its capacity. This elevated system allowed the roots to grow down through the tube into the soil and maintained the foliage high enough to provide space for pegging chambers. Twenty-five g of a 15:30:15 (N-P-K) fertilizer formulation was applied to each plant 15 days after planting. The plants were inoculated with Rhizobium leguminosarum Frank 21 days after planting, and 9 g of gypsum was added to the soil under each plant at flowering time (approximately 4 weeks after planting). All plants were sprayed to run off with Kelthane (Kelthane EC, 18.5%, 1.5 ml/liter) to control mites and monthly with triphenyl tin hydroxide (2.5g/liter) to reduce the incidence of Cercospora leaf spot.

For studies with pods attached to plants, pegs were surface disinfected with 1.0% sodium hypochlorite and

rinsed with sterile tap water. The pegs were then introduced into 50-ml test tubes that were covered with parafilm and aluminum foil and contained either autoclaved vermiculite (10% moisture v/v and 0.1 g of gypsum per tube) or soil (10% moisture v/v and 0.1 g of gypsum per tube). Pegs disinfected in the same way were also induced to grow in 200 ml polystyrene cups containing autoclaved soil.

Interactions of F. solani, P. myriotylum, and M. arenaria on attached peanut pods. Soil was infested by aseptically mixing eight V-8 juice broth cultures of each fungus into 1200 g of soil with an electric hand mixer. Sterile distilled water was added to the soil to give a final water content of 10% (w/w). After the infested soil had been maintained at approximately 27 C for 15 days in 2-liter Mason jars, 15-g samples were removed from each jar for population evaluation of each pathogen. Soil samples were assayed for P. myriotylum on the pimaricin-vancomycin selective medium (PV) of Tsao and Ocana (39), and for F. solani on the PDATS medium described in the first part of this dissertation. Infested soil was then diluted with autoclaved soil to give the desired inoculum density of each fungus.

Meloidogyne arenaria eggs were extracted by the method of McClure et al. (26) from the roots of the tomato plants.

The number of eggs in aqueous suspension was determined by counting the number of eggs in a 210 mm^2 area for 12 samples. Known quantities of eggs were added to soil to establish desired nematode populations.

Twenty-six attached pods for each treatment were maintained for 9 wk in test tubes containing soil infested with each pathogen alone, paired combinations of pathogens, or all three pathogens. Infested soil was prepared to contain P. myriotylum at 10 propagules/g of soil (ppg), F. solani at 3000 ppg, or M. arenaria at 5 eggs/ml of soil.

Interaction of P. myriotylum and R. solani with attached and detached peanut pods. Approximately six pods from each of 35 polystyrene cups that had been grown for 6 wk in autoclaved soil were carefully removed from the soil and the attached pods introduced into seven cups containing non-infested soil for a control or into 28 cups with infested soil. Soil in 14 cups was directly infested with 200 oospores of P. myriotylum/g of soil, and soil in an additional 14 cups was directly infested with 10 sclerotia of R. solani/g of soil (inoculum production and soil infestation methods as described in part one of this dissertation). After 20 days, pods were removed from seven cups with P. myriotylum infested soil and placed in cups containing soil infested with R. solani. Half of the cups with pods exposed to R. solani for 20 days were transferred to cups containing P. myriotylum. All cups were maintained for an additional 20 days in the greenhouse.

For studies with detached pods, mature pods were removed from the autoclaved soil and five detached pods were placed in each of eight polystyrene cups for each treatment. The treatments consisted of non-infested soil for a control, soil infested with 200 oospores of P. myriotylum/g of soil, and soil infested with 10 sclerotia of R. solani/g of soil. The cups were covered with aluminum foil and maintained at 30 C in a growth chamber. After 4 days, pods in eight cups with P. myriotylum were transferred to cups containing soil infested with R. solani, and pods in half of the cups with R. solani were transferred to soil containing P. myriotylum. All cups were incubated at 30 C for an additional 4 days.

Interactions of F. solani, M. phaseolina, P. myriotylum, and R. solani at high and low inoculum densities with detached peanut pods. AFter 8 to 9 wk of growth in autoclaved soil or vermiculite, six mature peanut pods were detached and placed in each of four cups for each treatment. The treatments consisted of non-infested soil for a control and soil directly infested with factorial combinations of the following fungi at low and high inoculum densities: (1) M. phaseolina at low, 100 sclerotia/g of soil, or high 200 sclerotia/g of soil, inoculum densities; (2) R. solani at low, 1 sclerotium/g of soil, and high, 10 sclerotia/g of soil, inoculum densities; and (3) F. solani plus P. myriotylum at low, 1000 conidia/g of soil plus 100 oospores/g of soil, respectively, and high, 10,000 conidia/g of soil plus 1000 oospores/g of soil, respectively, inoculum densities. The cups were incubated at 30 C for 12 days.

Successive in vitro inoculations with detached pods. Mature pods from soil were surface disinfested with 1.0% sodium hypochlorite for 40 seconds, rinsed three times in sterile distilled water, and four pods were placed on each of 12 (48 h-old) cultures of one of the following fungi: F. solani, M. phaseolina, P. myriotylum, or R. solani. After 36 hr at 30 C in the dark, 12 pods were transferred from cultures of one fungus to 48 hr old cultures of each of the other three fungi for an additional 72 hr. The remaining 12 pods exposed to each fungus were transferred to fresh cultures of the same fungus with which they were originally inoculated.

Successive exposure of detached pods to fungal cultures and infested soil. After surface disinfection with 1.0% sodium hypochlorite and three rinses in sterile distilled water, 100 pods were placed in 25 Petri plates containing cultures of one of the following fungi: F. solani, P. myriotylum, R. solani, or T. viride. After 72 h at 30 C in the dark, 25 pods from cultures of each fungus were placed in 5 cups containing soil directly infested with one of the other fungi. The infested soil contained F. solani at 1000 conidia/g of soil, P. myriotylum at 200 oospores/g of soil, R. solani at 10 sclerotia/g of soil, or T. viride at 30,000 conidia/g of soil. The cups were covered with aluminum foil and maintained at 30 C for four days. The pods were then removed from the cups and placed in cups containing P. myriotylum at 200 oospores/g of soil at 30 C for an additional 4 days.

Disease evaluation. The evaluation of pod rot severity was based either on the percentage of pod rot or on an index ranging from 1 for healthy pods to 5 for completely blackened pods. Frequency of isolation of pathogenic fungi from pods was evaluated by surface disinfecting pods with 1.0% sodium hypochlorite, rinsing three times with sterile distilled water, and plating them on the following selective media: PV for P. myriotylum, GSC medium for R. solani described by Ko and Hora (20), and potato dextrose agar, acidified with lactic acid to pH 4.0, for the isolation of M. phaseolina , F. solani and T. viride.

Results

Interactions of F. solani, P. myriotylum, and M. arenaria with attached peanut pods. Significant percentages of peanut pod rot occurred only in soil containing P. myriotylum (Table 8). Synergistic interactions in pod rot were observed when P. myriotylum was combined with F. solani, M. arenaria or a combination of both pathogens.

The frequency of isolation of P. myriotylum was highest from pods exposed to a combination of P. myriotylum and M. arenaria, but was not significantly higher than that from pods exposed to P. myriotylum alone or to a combination of P. myriotylum, M. arenaria, and F. solani. Fusarium solani was isolated from at least 23% of the pods in all of the treatments, but was isolated from a significantly greater percentage of pods that were exposed to combinations of of F. solani plus P. myriotylum or M. arenaria than from

pods in soil with F. solani alone. The percentages of isolation of F. solani from pods from soil infested with P. myriotylum or M. arenaria without F. solani were not significantly different from the percentage of isolation of F. solani from pods exposed to soil infested with F. solani.

Interactions of P. myriotylum and R. solani with attached and detached peanut pods. In both attached and detached fruits, pod rot was less severe when pods were exposed successively to R. solani and then P. myriotylum than when pods were exposed to P. myriotylum alone (Tables 9, 10). The antagonistic effect of R. solani on P. myriotylum was also observed in attached pods exposed to P. myriotylum before R. solani (Table 9). This effect was not observed with detached pods (Table 10).

Symptoms of pod rot were not found on detached pods from soil infested only with R. solani, (Table 10), but with attached pods, pod rot was significantly greater with R. solani than the control (Table 9).

Pythium myriotylum was isolated only from attached pods recovered from soil infested with P. myriotylum alone (Table 9). With detached pods, P. myriotylum was isolated from 90 to 100% of the pods initially exposed to soil infested with P. myriotylum and from 50% of the pods initially exposed to R. solani (Table 10). Over 50 % of the attached or detached pods initially exposed to R. solani yielded the fungus, but R. solani was not isolated from pods successively exposed to P. myriotylum and then to R. solani.

Interactions of F. solani, M. phaseolina, P. myriotylum and R. solani at high and low inoculum densities with detached peanut pods. When pods grown in autoclaved soil were detached and placed in infested soil, the incidence of pod rot which occurred with the high inoculum levels of P. myriotylum plus F. solani was significanlty reduced in the presence of R. solani when low levels of M. phaseolina were present, but not when high levels of M. phaseolina were present (Table 11). A high percentage of pod rot also occurred with high levels of P. myriotylum plus F. solani and with low levels of P. myriotylum plus F. solani in combination with a high level of M. phaseolina and a low level of R. solani. An antagonistic effect was observed with all other combinations involving low levels of P. myriotylum plus F. solani. The percentages of infection with high levels of R. solani and M. phaseolina in the absence of P. myriotylum plus F. solani was not significantly greater than the control.

Pythium myriotylum was isolated only from detached pods from soil containing P. myriotylum plus F. solani in the absence of both R. solani and M. phaseolina (Table 11). Rhizoctonia solani was not isolated from pods exposed to soil with the following inoculum levels: low R. solani, low M. phaseolina, and high P. myriotylum plus F. solani; high R. solani, high M. phaseolina, and low P. myriotylum plus F. solani; and high R. solani, low M. phaseolina, and low P. myriotylum plus F. solani. The percentage of isolation

of M. phaseolina was consistently greater from pods from soil with high levels of the fungus than from pods from soil with low levels of sclerotia. Pods from soil containing R. solani and M. phaseolina yielded significantly fewer colonies of F. solani than pods from soil containing P. myriotylum plus F. solani without the other fungi.

When pods were grown in vermiculite prior to exposure to infested soil, significant percentages of pod rot occurred only in soil containing P. myriotylum and F. solani in the absence of R. solani and M. phaseolina (Table 12). Pythium myriotylum was recovered only from pods from the same treatments, and R. solani conversely, was isolated from all other treatments except the control.

Successive in vitro inoculations with detached peanut pods. The inoculation of peanut pods with P. myriotylum alone or the successive in vitro inoculation with P. myriotylum and other fungi resulted in significantly higher severity of pod rot than in non inoculated controls (Table 13). When pods were inoculated with R. solani prior to inoculation with P. myriotylum, however the severity of pod rot was not significantly different from the control.

Inoculation of pods with P. myriotylum before or after exposure of pods to other fungi resulted in a high frequency of isolation of P. myriotylum, except when it was preceded by R. solani. The frequency of isolation of R. solani was generally high but was reduced when it was preceded by P. myriotylum or F. solani. Fusarium solani usually was isolated

from 100% of the pods exposed to it, but 33% of control pods yielded the fungus and F. solani was not isolated from pods from the other treatments not exposed to it.

Successive exposure of detached peanut pods to fungal cultures and infested soil. Inoculation in vitro with F. solani, T. viride, or P. myriotylum followed by exposure in soil to F. solani, R. solani, T. viride, or P. myriotylum, and a final exposure period to P. myriotylum infested soil resulted in significantly higher severity of pod rot than in other treatments (Table 14). In vitro inoculation with R. solani followed by exposure in soil to F. solani, P. myriotylum, R. solani, or T. viride, and a final exposure to P. myriotylum infested soil resulted in a pod rot severity that was not significantly greater than that in the control. The exposure of pods to R. solani in soil, prior to P. myriotylum exposure did not result in reduction of pod rot severity if the initial in vitro inoculation was with F. solani, T. viride, or P. myriotylum. None of the treatments with individual pathogens alone except total inoculation with P. myriotylum resulted in significant levels of pod rot over that in the control.

The frequency of isolation of P. myriotylum was over 60% in all treatments in which it was included, except in most sequences in which exposure to P. myriotylum was preceded by exposure to R. solani. However, the sequence R. solani - P. myriotylum - P. myriotylum yielded 80% isolation of P. myriotylum. No P. myriotylum was isolated

after the sequence F. solani - R. solani - P. myriotylum, R. solani - T. viride - P. myriotylum. In the sequence of inoculation with R. solani - R. solani - P. myriotylum only 16% of the pods yielded P. myriotylum. Rhizoctonia solani was isolated from over 35% of pods in all treatments in which pods were inoculated with the fungus in vitro. No R. solani could be isolated from pods exposed to it in soil if they were first inoculated in vitro with T. viride or P. myriotylum. Only 4.7% of the pods inoculated with F. solani and exposed to R. solani and then P. myriotylum yielded R. solani. The frequency of isolation of F. solani from pods which were exposed to it was above 68% except when soil exposure to F. solani was preceded by in vitro inoculation with T. viride. Fusarium solani was isolated from 0 to 41% of the pods in treatments in which it had not been included. The frequency of isolation of T. viride from pods in treatments that included exposure to it varied from 52 to 100%, and ranged from 0 to 32% from pods in treatments that had not included artificial infestation.

Table 8. Incidence of pod rot and frequency of isolation of fungi from attached peanut pods grown in test tubes containing soil with defined inoculum densities[x] of pathogens established by diluting infested soil with autoclaved soil.

Treatment	Pod rot (%)	Frequency of isolation (%)	
		P. myriotylum	*F. solani*
Non-inoculated	0.0a	---[z]	37.0 a
M. arenaria	0.0a	---	52.0 b
F. solani	5.5a	---	77.7 b
F. solani + *M. arenaria*	7.6a	---	100.0 c
P. myriotylum	19.23 b	38.4 ab	53.8 b
P. myriotylum + *F. solani*	47.0 c	21.0 a	100.0 c
P. myriotylum + *M. arenaria*	50.0 c	54.0 b	22.7 a
P. myriotylum + *M. arenaria* + *F. solani*	46.0 c	26.9 ab	100.0 c

[x]The populations of pathogens were 10 propagules per gram of soil (ppg) of *Pythium myriotylum*; 3000 ppg of *Fusarium solani*; and 5 eggs/ml of soil of *Meloidogyne arenaria*.

[y]Any given pair of treatments (within vertical columns) followed by the same letter are not different at the 0.05 level of significance, compared by the contingency chi square test.

[z]No isolation attempted.

Table 9. Pod rot severity and frequency of isolation of fungi from attached peanut pods grown in autoclaved soil for 6 wk and then exposed for 20 day periods to soil directly infested with known quantities of reproductive structures of Pythium myriotylum or Rhizoctonia solani[w].

Treatment[x]		Pod rot index[y]	Frequency of isolation (%)	
Initial exposure	Second exposure		P. myriotylum	R. solani
Non-inoculated		1.0 a[z]	---	---
R. solani	R. solani	1.5 b	---	62.5
R. solani	P. myriotylum	1.6 b	0.0	52.5
P. myriotylum	R. solani	1.8 b	0.0	0.0
P. myriotylum	P. myriotylum	3.6 c	40.0	---

[w]Soil infested with P. myriotylum at 200 oospores/g of soil, and R. solani at 10 sclerotia/g of soil.

[x]Pods exposed to soil containing one fungus for 20 days and then moved to soil containing the same or other fungus for 20 days.

[y]Pod rot index: 1=healthy pod; 5=completely blackened pod.

[z]Values followed by the same letter are not different at the 0.05 level of significance by Duncan's Multiple Range Test.

Table 10. Pod rot severity and frequency of isolation of fungi from mature detached peanut pods initially grown in autoclaved soil for 9 wk, and then exposed to directly infested soil with known quantities of reproductive structures of Pythium myriotylum or Rhizoctonia solani[W].

Treatment[X]		Pod rot index[Y]	Frequency of isolation (%)	
Initial exposure	Second exposure		P. myriotylum	R. solani
Non-inoculated		1.0 a[Z]	0.0	0.0
R. solani	R. solani	1.0 a	0.0	70.0
R. solani	P. myriotylum	1.8 a	50.0	80.0
P. myriotylum	R. solani	3.6 b	100.0	0.0
P. myriotylum	P. myriotylum	4.0 b	90.0	0.0

[W]Soil infested with P. myriotylum at 200 oospores/g of soil, and R. solani at 10 sclerotia/g of soil.

[X]Pods exposed to soil containing one fungus for 4 days and then moved to soil containing the same or other fungus for 4 days.

[Y]Pod rot index: 1=healthy pod; 5=completely blackened pod.

[Z]Values followed by the same letter are not different at the 0.05 level of significance by Duncan's Multiple Range Test.

Table 11. Incidence of pod rot and frequency of isolation of fungi from detached mature peanut pods after 8 wk growth in autoclaved soil and 12 days of exposure to combinations of fungi at high and low inoculum levels[y] in soil directly infested with known quantities of the fungal reproductive structures

Inoculum level			% of pod rot	Frequency of isolation (%)			
Rhizoctonia solani	Macrophomina phaseolina	Pythium myriotylum + Fusarium solani		P. myriotylum	R. solani	M. phaseolina	F. solani
High	High	High	23.8 b[z]	0.0 a	33.3 b	18.7 b	38.0 b
Low	High	High	31.2 b	0.0 a	18.7 b	12.5 b	31.2 b
High	Low	High	12.5a	0.0 a	6.2a	4.5 a	37.5 b
Low	Low	High	12.5a	0.0 a	0.0a	6.2 a	56.2 b
High	High	Low	6.2a	0.0 a	0.0a	25.0 b	31.2 b
Low	High	Low	25.0 b	0.0 a	25.0 b	25.0 b	37.5 b
High	Low	Low	18.7a	0.0 a	0.0a	4.7a	62.5 b
Low	Low	Low	18.7a	0.0 a	12.5 b	0.0a	52.2 b
High	High	None	12.5a	0.0 a	12.5 b	18.7 b	0.0a
None	None	High	37.5 b	31.2 b	0.0a	0.0a	87.5 c
None	None	Low	31.2 b	25.0 b	0.0a	0.0a	93.7 c
None	None	None	0.0a	0.0a	0.0a	0.0a	12.5a

[y]Inoculum levels were R. solani, high=10 sclerotia/g of soil (sgs), and low=1 sgs, M. phaseolina, high=200sgs, and low=100sgs, P. myriotylum plus F. solani, high=1000 oospores plus 10000 conidia/g of soil, respectively, and low=100 oospores plus 1000 conidia/g of soil respectively.

[z]Any given pair of values in a column followed by the same letter are not different at the 0.05 level of significance compared with the contingency chi square test.

Table 12. Incidence of pod rot and frequency of isolation of fungi from detached peanut pods after 8 wk growth in autoclaved vermiculite and 12 days of exposure to combinations of fungi at high and low inoculum levels[y] in soil directly infested with known quantities of the fungal reproductive structures.

Inoculum level			% of pod rot	Frequency of isolation (%)	
Rhizoctonia solani	*Macrophomina phaseolina*	*Pythium myriotylum* + *Fusarium solani*		*P. myriotylum*	*R. solani*
High	High	High	11.2 a[z]	0.0 a	66.6 b
Low	High	High	22.2 a	0.0 a	100.0 b
High	Low	High	11.1 a	0.0 a	100.0 b
Low	Low	High	11.1 a	0.0 a	100.0 b
High	High	Low	0.0 a	0.0 a	77.7 b
Low	High	Low	0.0 a	0.0 a	100.0 b
High	Low	Low	0.0 a	0.0 a	77.7 b
Low	Low	Low	0.0 a	0.0 a	88.8 b
High	High	None	0.0 a	0.0 a	77.7 b
None	None	High	100.0 b	100.0 a	0.0 a
None	None	Low	77.7 b	44.4 a	0.0 a
None	None	None	0.0 a	0.0 a	0.0 a

[y] Inoculum levels were *R. solani*, high=10 sclerotia/g of soil (sgs), and low=1sgs, *M. phaseolina*, high=200 sgs, and low=100 sgs, *P. myriotylum* plus *F. solani*, high=1000 oospores plus 10000 conidia/g of soil, respectively, and low=100 oospores plus 1000 conidia/g of soil, respectively.

[z] Any given pair of values in a column followed by the same letter are not different at the 0.05 level of significance compared with the contingency chi square test.

Table 13. Influence of successive 36 h exposures of detached peanut pods to Petri dish cultures of fungi on pod rot severity, and frequency of isolation of fungi.

Inoculation		Pod rot index[y]	Frequency of isolation (%)			
Initial exposure	Secondary exposure		Pythium myriotylum	Rhizoctonia solani	Macrophomina phaseolina	Fusarium solani
P. myriotylum	F. solani	5.0 a[z]	100.0	---	---	100.0
F. solani	P. myriotylum	4.3 ab	66.5	---	---	100.0
P. myriotylum	M. phaseolina	4.2 ab	78.5	---	0.0	0.0
P. myriotylum	R. solani	4.1 ab	90.0	5.0	---	0.0
P. myriotylum	P. myriotylum	4.1 ab	86.3	---	---	0.0
M. phaseolina	P. myriotylum	3.0 b	100.0	---	7.1	0.0
F. solani	M. phaseolina	2.3 c	---	---	0.0	100.0
F. solani	R. solani	2.3 c	---	23.6	---	100.0
R. solani	F. solani	2.1 c	---	45.5	---	78.5
F. solani	F. solani	2.1 c	---	---	---	100.0
R. solani	P. myriotylum	1.9 c	41.5	95.5	---	0.0
R. solani	M. phaseolina	1.8 c	---	78.5	52.2	0.0
M. phaseolina	R. solani	1.4 c	---	71.4	37.9	0.0
R. solani	R. solani	1.2 c	---	92.8	---	0.0
M. phaseolina	F. solani	1.2 c	---	---	16.5	100.0
M. phaseolina	M. phaseolina	1.0 c	---	---	83.0	0.0
None	None	1.0 c	0.0	0.0	0.0	33.3

[y]Pod rot index: 1=healthy pod; 5= completely blackened pod.

[z]Values followed by the same letter are not different at the 0.05 level of significance by Duncan's Multiple Range Test.

Table 14. Effect of in vitro inoculation of detached peanut pods for 72 h followed by two successive 4 day exposures of pods to soil infested with various fungal pathogens[x] on severity of pod rot and frequency of isolation of fungi from mature pods initially grown in autoclaved soil.

In vitro inoculation	First soil exposure	Second soil exposure
F. solani	*T. viride*	*P. myriotylum*
T. viride	*F. solani*	*P. myriotylum*
F. solani	*P. myriotylum*	*P. myriotylum*
F. solani	*F. solani*	*P. myriotylum*
F. solani	*R. solani*	*P. myriotylum*
T. viride	*P. myriotylum*	*P. myriotylum*
P. myriotylum	*T. viride*	*P. myriotylum*
None	*P. myriotylum*	*P. myriotylum*
P. myriotylum	*P. myriotylum*	*P. myriotylum*
P. myriotylum	*F. solani*	*P. myriotylum*
T. viride	*T. viride*	*P. myriotylum*
T. viride	*R. solani*	*P. myriotylum*
P. myriotylum	*R. solani*	*P. myriotylum*
R. solani	*F. solani*	*P. myriotylum*
R. solani	*P. myriotylum*	*P. myriotylum*
R. solani	*R. solani*	*P. myriotylum*
R. solani	*T. viride*	*P. myriotylum*
F. solani	*F. solani*	*F. solani*
R. solani	*R. solani*	*R. solani*
T. viride	*T. viride*	*T. viride*
None	None	None

[x]Population of fungi were *Pythium myriotylum*, 200 oospores/g of soil; *Rhizoctonia solani*, 10 sclerotia/g of soil; *Fusarium solani*, 1000 conidia/g of soil; and *Trichoderma viride* 30,000 conidia/g of soil.

Pod rot index[y]	Frequency of isolation (%)			
	P. myriotylum	R. solani	F. solani	T. viride
4.64 a[z]	68.9	---	86.9	52.1
4.60 a	90.4	---	19.0	80.9
3.27 a	77.2	---	81.8	0.0
4.15 a	90.9	---	81.8	0.0
4.14 a	0.0	4.7	100.0	14.2
4.10 a	68.1	---	40.9	68.1
4.08 a	100.0	---	0.0	59.0
3.96 a	100.0	---	8.0	12.0
3.96 a	100.0	---	8.0	12.0
3.90 a	95.4	---	77.2	0.0
3.82 a	86.3	---	13.6	59.0
3.80 a	0.0	0.0	12.5	62.5
3.64 a	60.8	0.0	34.7	17.3
2.09 b	0.0	45.4	68.1	9.0
1.91 b	80,0	35.0	40.0	10.0
1.85 b	16.0	60.0	28.0	32.0
1.80 b	0.0	59.0	18.1	63.6
2.16 b	---	---	100.0	0.0
1.33 b	---	60.0	40.0	40.0
1.23 b	---	---	---	100.0
1.23 b	---	---	16.0	16.0

[y]Pod rot index: 1=healthy; 5=completely blackened pod.

[z]Values followed by the same letter are not different at the 0.05 level of significance by Duncan's Multiple Range Test.

Discussion

The results of this study provide information on the complex role played by Pythium myriotylum in pod rot of peanut. Pythium myriotylum interacted synergistically with both Fusarium solani and Meloidogyne arenaria in pod rot. Although a synergistic interaction between M. arenaria and P. myriotylum has not been reported in the literature, Minton and Jackson (32) reported that the presence of M. arenaria increased the total number of fungi isolated from pods and the total number of blemishes on pods. A synergistic interaction between P. myriotylum and M. arenaria in damping-off of peanut seedlings was reported in Part 1 of this dissertation. The synergistic interaction between P. myriotylum and F. solani has been reported previously by Frank (7), who suggested that other organisms may function similarly to F. solani in interactions with P. myriotylum. Other reports have since indicated that soil mites and insects interact with P. myriotylum and other fungi in pod rot (3, 36). Porter and Smith (35) for example, found that pod rot disease was greatly enhanced in soil infested with P. myriotylum if the southern corn rootworm was present.

Although P. myriotylum and Rhizoctonia solani are considered to be two of the most important pathogens involved in peanut pod rot, interactions between these two

fungi have received little critical attention. Garren (10) observed antagonism between R. solani and P. myriotylum and found that indigenous populations of P. myriotylum dominated over introduced R. solani in causing pod rot. In fields where both pathogens are present, one fungus may be isolated more frequently from rotted pods one year, and the other fungus may predominate another year (Mitchell, D. J., unpublished data), (8, 40). The results of this study, as well as those of Garren (11), indicate that damage is much more severe with P. myriotylum than with R. solani. The isolate of R. solani used in this experiment caused a minimal but significant amount of rot in attached pods (Table 9), but did not cause damage to detached pods in any of the other experiments (Tables 10 through 14). Furthermore, R. solani acted as an antagonist to P. myriotylum because the colonization of attached or detached pods by R. solani prevented or reduced the development of pod rot in pods later exposed to P. myriotylum in vitro or in soil (Tables 9 through 14). Pod rot caused by P. myriotylum was not reduced in the presence of R. solani if the detached pods were inoculated with P. myriotylum before R. solani (Tables 10, 13, 14). The antagonistic effect of R. solani on P. myriotylum in pods formed in soil was nullified when a high inoculum density of Macrophomina phaseolina was present (Table 11); this effect, however, was not observed in pods produced in autoclaved vermiculite before exposure to the fungi (Table 12).

The frequency of isolation of P. myriotylum and R. solani from rotted pods also demonstrated the antagonistic interaction between these two fungi. Although P. myriotylum was isolated from 40% of the attached pods from soil infested with it alone, it was not isolated from any of the pods exposed to soil infested with both fungi (Table 9). Pythium myriotylum generally could not be isolated or was isolated at much lower frequencies from detached pods that were exposed to R. solani in vitro or in soil prior to exposure to P. myriotylum (Tables 10 through 14). Rhizoctonia solani, conversely, was isolated much less frequently or could not be isolated from attached or detached pods exposed first to P. myriotylum (Tables 9, 10, 13, 14). When fungi other than P. myriotylum and R. solani were present, several trends with isolation frequencies of the latter two fungi were observed. No P. myriotylum was isolated from pods exposed to combinations of it plus F. solani or Trichoderma viride. Rhizoctonia solani was isolated at a high frequency from pods in treatments which included exposure to R. solani in vitro and then soil exposures with F. solani or T. viride before exposure to P. myriotylum; few or no pods contained R. solani if pods were colonized in vitro with F. solani or T. viride before exposure to R. solani. High amounts of pod rot occurred in treatments with P. myriotylum when the pods were colonized with T. viride or F. solani before R. solani.

The antagonistic effect of R. solani on P. myriotylum occurred when it colonized pods before F. solani or T. viride, and in such cases, pod rot was not significantly greater than in the control pods. Thus, the severity of pod rot and the frequency of isolation of fungi from pods are strongly influenced by sequence of exposure of pods to the various fungi and are especially influenced by the earliest invaders of the pod. Although Garren (12) did not specifically indicate which fungi might be antagonistic to P. myriotylum, he did observe that P. myriotylum survived longer in soil in which it was not indigenous, than in soil to which it was not native, and suggested that organisms antagonistic to P. myriotylum were present in the native soil. Garren (9) also recognized that antagonistic organisms colonizing pods could be part of the natural barrier to invasion by phytopathogenic fungi. Flowers and Littrell (6, 24) reported that one isolate of R. solani was antagonistic to Pythium aphanidermatum (Edson) Fitz. in culture and that P. aphanidermatum could not be recovered from water suspensions or soil to which both fungi had been added.

The points discussed above also provide possible explanations for the difficulty encountered by Garren (8) and others (7, 22) to isolate certain fungi from pods which had symptoms typical of those related to respective fungal pathogens. Pythium myriotylum, in this study for

example, was not isolated from rotted pods with typical symptoms associated with P. myriotylum that were colonized in vitro by F. solani or T. viride and then exposed to R. solani and P. myriotylum in soil (Table 14). Trichoderma viride was isolated in low numbers from pods except those that were colonized by it before exposure to other fungi, (Table 14), but it is readily apparent how this fungus could be associated with pod rot if observations are based on fungal isolations. Finally neither P. myriotylum nor R. solani could be isolated from attached pods exposed to P. myriotylum before R. solani (Table 9).

Unless gnotobiotic conditions are used to evaluate interactions of fungi in peanut pods, F. solani and T. viride are consistently present as inhabitants of the endogeocarp or geocarposphere (7, 8, 9, 22). When pathogenicity studies are conducted to evaluate fungi which are ubiquitously present, it is thus important to clearly define the inoculum levels prepared with reference to background populations of the respective fungi and relate these factors to the final isolations of fungi from pods.

Although the possibility of using avirulent isolates of R. solani to protect peanut pods from damage by P. myriotylum is of interest, the specific environmental conditions under which P. myriotylum is biologically controlled but under which pods are not damaged must first be ascertained. Wills and Moore (42) have reported several isolates of R. solani from pods that were nonpathogenic to

peanut seedlings, but no reports on the range of pathogenicity of isolates of R. solani to pods were found by the author.

LITERATURE CITED

1. Bell, D. K., and N. A. Minton. 1973. Postemergence damping-off of peanut plants caused by Pythium myriotylum. Phytopathology 63:1544-1545.

2. Bell, D. K., N. A. Minton, and B. Doupnik, Jr. 1970. Infection of peanut pods by Aspergillus flavus as affected by Meloidogyne arenaria and length of curing time. Phytopathology 60:1284 (Abstr.).

3. Beute, M. K. 1974. A quantitative technique for the extraction of soil-inhabiting mites (Acarina) and springtails (Collembola) associated with pod rot of peanut. Phytopathology 64:571-572.

4. Edmunds, J. E., and W. F. Mai. 1966. Population increase of Pratylenchus penetrans in alfalfa and celery roots infected with Trichoderma viride. Phytopathology 56:1320-1321.

5. Escobar, C., M. K. Beute, and J. L. Lockwood. 1967. Possible importance of Pythium in root rot of peas. Phytopathology 57:1149-1151.

6. Flowers, R. A., and R. H. Littrell. 1973. Influence of Rhizoctonia solani on population densities of Pythium aphanidermatum in soil. 2nd Internat. Congr. of Plant Pathol., Minneapolis, Minn., Abstr. No. 1058.

7. Frank, Z. R. 1972. Pythium myriotylum and Fusarium solani as cofactors in a pod-rot complex of peanut. Phytopathology 62:1331-1334.

8. Garren, K. H. 1963. Peanut (groundnut) microfloras and pathogenesis in peanut pod rot. Phytopathol. Z. 55:359-367.

9. Garren K. H. 1967. Relation of several pathogenic organisms, and the competition of Trichoderma viride to peanut pod breakdown. Plant Disease Reporter 51:601-605.

10. Garren K. H. 1970. Antagonisms between indigenous Pythium myriotylum and introduced Rhizoctonia solani and peanut pod breakdown. Phytopathology 60:1292 (Abstr.).

11. Garren, K. H. 1970. Rhizoctonia solani versus Pythium myriotylum as pathogens of peanut pod breakdown. Plant Disease Reporter 54:840-843.

12. Garren, K. H. 1971. Persistence of Pythium myriotylum in soils. Phytopathology 61:596-597.

13. Garren, K. H., and C. R. Jackson. 1973. Peanut diseases. In. Peanuts-Culture and Uses. (A symposium.) The American Peanut Research and Education Association Inc. Stone Printing Co. Virginia. pp. 429-494.

14. Gay, J. D. 1969. Effect of temperature and moisture on snap bean damping-off caused by three isolates of Pythium myriotylum. Plant Disease Reporter. 53:707-709.

15. Hanlin, R. T. 1973. The distribution of peanut fungi in the southeastern United States. Mycopathologia et Mycologia applicata 49:227-241.

16. Jackson, C. R. 1965. Reduction of Sclerotium bataticola infection of peanut kernels by Aspergillus flavus. Phytopathology 55:934.

17. Jackson, C. R., and D. K. Bell. 1969. Diseases of peanut (groundnut)caused by fungi. Georgia Agric. Exp. Sta. Res. Bull. 56:137 pp.

18. Johnson, A. W., and R. H. Littrell. 1970. Pathogenicity of Pythium aphanidermatum to chrysanthemum in combined inoculations with Belonolaimus longicaudatus or Meloidogyne incognita. J. of Nematol. 2:255-259.

19. Kerr, A. 1962. The root rot-Fusarium wilt complex of peas. Aust. J. Biol. Sci. 16:55-69.

20. Ko, W., and F. K. Hora. 1971. A selective medium for the quantitative determination of Rhizoctonia solani in soil. Phytopathology 61:707-710.

21. Kraft, J. M., and D. D. Roberts. 1969. Influence of soil, water and temperature on the pea root rot complex caused by Pythium ultimum and Fusarium solani f. sp. pisi. Phytopathology 59:149.

22. Kranz, J. and E. Pucci. 1963. Studies on soil-borne rots of groundnuts (Arachis hypogaea). Phytopathol. Z. 47:101-112.

23. Littrell, R. H., and A. W. Johnson. 1969. Pathogenicity of Pythium aphanidermatum to chrysanthemum in combined inoculations with Belonolaimus longicaudatus or Meloidogyne incognita. Phytopathology 59:115 (Abstr.).

24. Littrell, R. H., and R. A. Flowers. 1973. Isolates of Rhizoctonia solani non-antagonistic and antagonistic to Pythium aphanidermatum. 2nd Internat. Congr. of Plant Pathol., Minneapolis, Minn., Abstr. No. 1061.

25. McCarther, S. M., and R. H. Littrell. 1968. Pathogenicity of Pythium myriotylum to several grass and vegetable crops. Plant Disease Reporter. 52:179-183.

26. McClure, T., H. Kruk, and I. Misaghi. 1973. A method for obtaining quantities of clean Meloidogyne eggs. J. Nematol. 5:230.

27. Melendez, P. L., and N. T. Powell. 1969. The influence of Meloidogyne on root decay in tobacco caused by Pythium and Trichoderma. Phytopathology 59:1348 (Abstr.).

28. Melendez, P. L., and N. T. Powell. 1970. The Pythium-root knot nematode complex in flue-cured tobacco. Phytopathology 60:1303 (Abstr.).

29. Meyer, W. A., Sinclair, J. B., and M. N. Kharé. 1973. Biology of Macrophomina phaseoli in soil studied with selective media. Phytopathology 63:613-620.

30. Minton, N. A. 1963. Effects of two populations of Meloidogyne arenaria on peanut roots. Phytopathology 53:79-81.

31. Minton, N.A., and C. R. Jackson. 1967. Effects of Meloidogyne arenaria on the incidence of Aspergillus flavus in peanuts. Nematologica 13:146 (Abstr.).

32. Minton, N. A., and C. R. Jackson. 1967. Invasion of peanut pods by Aspergillus flavus and other fungi in the presence of root knot nematode. Oleagineaux 22:543-546.

33. Oyekan, P. O., and J. E. Mitchell. 1972. The role of Pratylenchus penetrans in the root rot complex of canning peas. Phytopathology 62:369-373.

34. Porter, D. M. 1970. Peanut wilt caused by Pythium myriotylum. Phytopathology 60:393-394.

35. Porter, D. M., and J. C. Smith. 1974. Fungal colonization of peanut fruits as related to southern corn rootworm injury. Phytopathology 64:249-251.

36. Powell, N. P. 1971. Interactions between nematodes and fungi in disease complex. Annual Review of Phytopathology 9:253-274.

37. Steiner, G. W., and R. D. Watson. 1965. Use of surfactants in the soil dilution plate count method. Phytopathology 55:728-730.

38. Thames, W. H., Jr., and B. C. Langley. 1964. Chemical control of Meloidogyne arenaria on Spanish peanuts in Texas. Phytopathology 54:626-627. (Abstr.)

39. Tsao, P. H., and C. Ocana. 1969. Selective isolation of species of Phytophthora from natural soils on an improved antibiotic medium. Nature 223:636-638.

40. Van Schaik, P. H., Garren, K. H., and D. M. Porter. 1972. Potential sources of resistance to pod breakdown in peanuts. American Peanut Research and Education Association Inc. 4:14-17.

41. Wells, T. R., W. A. Kreutzer, and D. L. Lindsey. 1972. Colonization of gnotobiotically grown peanuts by Aspergillus flavus and selected fungi. Phytopathology 62:1238-1242.

42. Wills, W. H., and L. D. Moore. 1973. Pathogenicity of Rhizoctonia solani and Pythium myriotylum from rotted pods to peanut seedlings. Plant Disease Reporter 57:578-582.

BIOGRAPHICAL SKETCH

Roberto Garcia was born in Mexico City, Mexico, on June 24, 1944. He received the degree of Bachelor of Science in biology at Universidad Nacional Autonoma de Mexico in 1967. In 1968, he received the Master of Science degree at Colegio de Post-Graduados, Escuela Nacional de Agricultura, Mexico, with a major in Plant Pathology. From 1969 to 1970, he was head Plant Pathologist of the Agricultural Experiment Station of Chontalpa, Tabasco, Mexico. In 1971, he was professor at the Colegio Superior de Agricultura Tropical, in the same state. In Spring and Summer of 1972 he was a visiting professor at the University of Hawaii, Plant Pathology Department. In the Fall quarter of 1972, he began studies toward the degree of Doctor of Philosophy at the University of Florida. He is a member of the American and of the Mexican Phytopathological Society. He has accepted the position of professor with Colegio Superior de Agricultura Tropical, Tabasco, Mexico.

In 1971 he married the former Ana Maria H. Roman, in Mexico City, Mexico.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David J. Mitchell
Chairman
Assistant Professor of
Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert T. McMillan, Jr.
Associate Professor of
Plant Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Laurence H. Purdy
Professor of Plant
Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

for Norman C. Schenck
Professor of Plant
Pathology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Grover C. Smart, Jr.
Professor of Entomology and Nematology

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1974

Dean, College of Agriculture

Dean, Graduate School